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Integrated management of cyst nematodes in oilseed rape

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1. ABSTRACT

Oilseed rape (OSR) production in the UK has increased markedly since the 1970s. This has led to reduced rotation lengths with potential for increased damage and yield loss by the two OSR cyst nematode species; the brassica cyst nematode (*Heterodera cruciferae*) and the sugar beet cyst nematode (*H. schachtii*). This project aimed to: assess the distribution and potential yield losses caused by cyst nematodes in OSR, provide management advice to minimise current and future crop losses and investigate the effect of temperature on *H. schachtii* development.

A survey for the two cyst nematode species was conducted in OSR-growing areas of the UK, whilst glasshouse and polytunnel experiments were conducted to investigate the host status of five of the most popular UK OSR cultivars (cvs), and the relationship between initial population densities of *H. schachtii*, *H. cruciferae* and yield. Water bath and outdoor pot experiments were conducted to investigate the effect of temperature on the life cycle of *H. schachtii*, and the number of generations completed by *H. schachtii* on OSR during the growing season.

Cyst nematode species *H. schachtii* (70%), the cereal cyst nematode (*H. avenae* complex) (19%) and the potato cyst nematode (PCN) (*Globodera pallida*) (10%) were detected in 14% of the 221 survey samples, whilst 11% of the samples contained a mixture of both *H. schachtii* and *G. pallida*. The population densities were generally low with 85% of the samples having less than 10 eggs g⁻¹ of soil, whilst *H. cruciferae* was not detected in any of the samples.

The winter OSR cvs Flash and DK Cabernet were more susceptible to cyst nematode damage than cvs ES Astrid, Castille and Catana respectively.

Temperature influenced the development of *H. schachtii* with the duration of the life cycle ranging between three and six weeks. The optimum temperature for development ranged between 20.5 and 32.2°C, whilst at least two generations of *H. schachtii* were completed during the growing season.

These results indicate that, given the high potential for nematode multiplication at low population densities and the rising UK soil temperatures, coupled with the low resistance of current OSR cvs, agronomic practices may lead to build-up of high cyst nematode population densities in the future.

2. INTRODUCTION

The area under OSR production in the UK has been progressively increasing since the early 1970s, and has varied from 6,500 ha (Bunting, 1984; Evans and Russell, 1993) to the current 705,000 ha (DEFRA, 2011). The latter hectareage accounts for 2.8 million tonnes of UK OSR production (DEFRA, 2011). Oilseed rape (12.9%) has become the third most important crop cultivated in the UK after winter wheat (47%) and spring barley (13%), and is worth £400 million per annum (Twining and Clarke, 2009). The driving factors for the increasing hectareage of OSR include: the high financial returns from the crop, the increasing demand for the crop as a source of vegetable cooking oil and the Renewable Transport Fuel Obligation (RTFO) (EU directive 2003/30/EC), among other uses (Anon, 2011). The RTFO initially required the substitution of transport fuels by biofuel by 2% prior to 2005 and 5.75% by 2010 (DEFRA, 2003). The directive aimed to reduce the use of fossil fuels and lower the carbon foot print (CFP) from the transport sector in order to lessen environmental degradation (De Vries *et al.*, 2008). The RTFO was later replaced by a new EU directive (2009/28/EC) targeting 12% substitution of transport fuels with biofuels by 2020 (McMeekin, 2010). As a result, there has been an increasing demand for OSR which has led to a reduction in rotation lengths and a subsequent build-up of pests and diseases and increased disease-associated yield loss (Berry and Spink, 2006). Rotation lengths have reduced from one year of OSR in six of cereals and/or pulses which was practised in the mid 1980s (Evans, 1984), to less than one year in two currently practised by some growers in the UK (S. Kakaire, personal observation).

Evans (1984) predicted an increase in crop losses resulting from cyst nematode damage following an increase in OSR hectareage. Oilseed rape cyst nematodes are presumed to play a key role in OSR yield decline and the duration of their life cycles is temperature-dependant. The rising soil temperatures in the UK (Carter *et al.*, 2000; Subedi and Fullen, 2009) could shorten generation times of these cyst nematodes which could potentially increase their damage to OSR. Agronomic factors including poor timing of drilling, insufficient pest and disease management and weeds, among others, have also adversely affected OSR yield. As a result, mean OSR yields have remained sub-optimal since the 1980s despite the potential yield of winter cvs being inherently higher (6.5 t ha^{-1}) (Berry and Spink, 2006; Mills *et al.*, 2009).

2.1. Symptoms of cyst nematode damage on OSR

Oilseed rape plants infected with *H. cruciferae* and *H. schachtii* manifest patchy growth (Sykes and Winfield, 1966) and lateral root proliferation (Harris and Evans, 1988), which appear in late October to early November in winter crops (Bowen, 1988). In severe cases, death of the plants may eventually occur (Harris and Winfield, 1985; Winfield, 1992) (Figure 1).



Figure 1. Field symptoms of cyst nematode infection in oilseed rape: patchy growth, plant death and lateral root proliferation.

2.2. Objectives of the research

- To assess UK distribution and yield losses caused by cyst nematodes in OSR and provide integrated management advice to minimise current and future crop losses
- To establish the extent of nematode resistance/tolerance in five of the most popular UK OSR cvs
- To investigate the effect of temperature on cyst nematode development

2.3. Integrated management strategies for cyst nematodes

Understanding the biology, physiology and biochemistry of plant-parasitic nematodes (PPNs) forms the basis for the determination of appropriate control strategies (Perry, 1994). Modern management of cyst nematodes involves a combination of methods including: nematicides, resistant and/or tolerant cvs, rotation with non-host crops, trap cropping, biocontrol and biofumigation using mustards and oil radish (Hillnhütter *et al.*, 2011). The Integrated Pest Management (IPM) approach is employed to offset limitations of individual management methods and involves the simultaneous application of two or more methods with complementary effects which ultimately produce a synergistic reduction in yield loss and nematode Pi's which could infect subsequent crops (Trudgill *et al.*, 2003).

2.3.1. Chemical nematicides

Chemical nematicides have been widely used in the management of PPNs, especially the potato cyst nematodes (PCN), for many years. However, as a result of increasing environmental concerns, their use within the European Union is being restricted under EU directives 91/414/EEC (Europa, 1991) and EC 1107/2009. No chemical nematicides have been approved for use in the management of OSR cyst nematodes which implies that non-chemical alternatives have to be explored (Caswell-Chen *et al.*, 1992). However, since potatoes are grown in rotation with OSR, Metam sodium (Noling, 1997) and other nematicides, which are used in PCN management, may inadvertently control OSR cyst nematodes.

2.3.2. Resistance/tolerance

Resistance refers to the ability of a plant to prevent or reduce nematode invasion, development or multiplication (Trudgill, 1986; 1991), whilst tolerance is the inherent potential of plants to compensate for the damage caused by cyst nematode infection, by maintaining good plant growth and yield (Evans and Haydock, 1990; Haydock and Evans, 1998). The use of cultivar resistance/tolerance as management measures for PPNs is a good strategy as it is cost effective, does not require training or specialised machinery/equipment, has no known toxic effects to the environment and helps reduce rotation lengths in cropping cycles (Trudgill, 1991).

2.3.3. Crop rotation

The principle of cyst nematode management by crop rotation is based on the natural decline of the nematodes in the absence of host plants. If the rate of annual cyst nematode population density decline is constant, accurate prediction of the length of the rotation necessary to lower the population density to below economic threshold level is possible. This is because a high population decline would require a longer crop rotation period with non-host plants to reduce the population density to below the tolerance limit (Griffin, 1988). Crop rotation is an economically feasible and environmentally-friendly option for cyst nematode management, and allows incorporation of non-host plants into the cropping sequence (Schmitt and Ferris, 1998). However, this method requires multiple seasons of growing non-host crops before host crops are planted again on the same fields. Knowledge of the nematode host range is essential for crop rotation to be effective. Maximum benefit derived from non-host crops may necessitate leaving nematode-infested land under fallow for long periods (Haydock and Evans, 1998); however, in some cases, nematodes can adapt to

survival in the absence of host plants (Schmitt and Ferris, 1998). Care should also be taken to remove all potential host plants including volunteers and weeds if maximum nematode decline is to be attained through crop rotation (Haley, 2004). However, nematode decline rates vary between sites depending on environmental and other factors as well as nematode species.

2.3.4. Biological control

Since many of the methods used against cyst nematodes are either not economically feasible or are harmful to the environment, biological control using natural enemies of the nematodes is a suitable alternative (Mennan *et al.*, 2005). Biological control has great potential for managing cyst nematodes in many agricultural systems whereby micro-organisms that occur naturally in the soil facilitate the rate of population density decline by parasitising cyst nematode eggs or preventing females from laying eggs (Mennan *et al.*, 2005). Fungal parasites such as *Pochonia chlamydosporia* (Kerry, 1987) and *Fusarium oxysporum* (Mennan *et al.*, 2005), collembolan (springtails) cyst predators (Murphy and Doncaster, 1957) and bacterial parasites such as *Pasteuria nishizawae* and fluorescent *Pseudomonas* spp. (Aksoy and Mennan, 2004) have been implicated in the natural decline of cyst nematodes. Natural decline in cyst nematode population densities can also occur due to in-egg mortality as a result of partial hatch of the second stage juveniles (J2) following brief exposure to HPRD, which become vulnerable to unfavourable environmental conditions (Forrest, 1989; Haley, 2004).

2.3.5. Trap cropping

Trap crops stimulate large scale cyst nematode J2 to hatch but inhibit syncytia establishment hence the nematodes fail to multiply and eventually die (Fleming *et al.*, 1998). Trap cropping involves the use of cyst nematode host plants to elicit J2 hatch and the plants are later destroyed before the nematodes complete their life cycles in the roots (Holgardo and Magnusson, 2010). Trap crops have been reported to reduce nematode population densities by 80% (Haydock and Evans, 1998). Oil radish and mustards have proved effective in reducing *H. schachtii* population densities in the field (Stefanovska and Pidlisnyuk, 2009), whilst sticky nightshade has been reported to reduce PCN population densities by 77% (Scholte and Vos, 2000). However, the major limitation to the widespread use of sticky nightshade is the need to grow the plants for an entire season if they are to be effective (Timmermans *et al.*, 2006). This is not very popular with growers as it is not economically feasible to keep productive land under a non-economic crop for an entire growing season.

2.3.6. Biofumigation

Biofumigation is increasingly being used because of its potential as an effective method in the management of soil-borne pests and diseases (Matthiessen and Kirkegaard, 2006). Biofumigation is the suppression of soil-borne plant pests and pathogens by compounds released when plant residues are hydrolysed (Kirkegaard *et al.*, 1993). Oilseed brassica species including OSR and other cruciferous plants produce secondary compounds called glucosinolates (GSL) (Underhill, 1980). Glucosinolates, when exposed to the enzyme myrosinase during tissue damage, become hydrolysed to yield nitriles, epithionitriles (at low pH), thiocyanates and isothiocyanates (at high pH), which are toxic to soil micro-organisms (Fahey *et al.*, 2001). However, the effectiveness of GSL depends on the type of brassica species used and the timing of application. The toxic potential of biofumigant crops is said to be greatest in young tissues (Halkier and Gershenson, 2006) and at flowering time (Bellostas *et al.*, 2007). Lazzeri *et al.* (1993) reported that between 0.05 and 0.5 mg ml⁻¹ of GSL in the presence of myrosinase enzyme controlled *H. schachtii* in sugar beet in Italy, whilst Tylka *et al.* (1997) reported irreversible inhibition of the soybean cyst nematode *H. glycines* J2 hatch in the USA on exposure to GSL. Buskov *et al.* (2002) observed 100% mortality of *G. rostochensis* J2 within 16 hours *in vitro* when myrosinase was mixed with 1 mg l⁻¹ of phenethylglucosinolate at a pH of 6.5, whilst Aires *et al.* (2009) demonstrated that PCN suppression in soil was dependent on total GSL concentration and the type of brassica species extract used. They observed that a total GSL concentration of 0.2 μ moles 100 g⁻¹ dry weight was sufficient to cause a significant reduction in the number of new *G. rostochensis* cysts which were formed on potatoes 29 days after incorporation of the extracts. Lord *et al.* (2011) reported that three Indian mustard (*Brassica juncea*) lines (Nemfix, Fumus, and ISC I99) containing high concentrations of 2-propenylglucosinolate caused over 95% mortality of encysted eggs of *G. pallida* in polyethylene-covered soil after incorporation.

2.3.7. Plant extracts

Plant extracts have been in use for a long time (Cowan *et al.*, 1999), but their popularity in plant pest and disease management has been increasing as a result of environmental concerns about the use of conventional pesticides. Plant extracts and essential oils have shown potential in the management of PPNs (Akhtar and Mahmood, 1994). However, the large scale use of plant extracts has been hampered by a number of limitations including: the cost of purification, transportation and application of large volumes of the extracts which are normally required to achieve the desired lethal concentrations (Chitwood, 2002). Some commonly used plant extracts include: marigolds (*Tagetes* spp.) (Chitwood, 2002), neem

(*Azadirachta indica*) (Bhattacharya and Goswami, 1987), pyrethrum (*Chrysanthemum cinerariaefolium*) (Isman, 2006), dazitol (*Allyl isothiocyanate*) (Martin *et al.*, 2007) and garlic (*Allium sativum* L.) (Block *et al.*, 1992). Danquah (2012) showed that concentrations of garlic extract less than 137.6 $\mu\text{l ml}^{-1}$ stimulated *G. pallida* hatch by approx. 26% above the potato root leachate control.

2.3.8. Soil solarisation

Soil solarisation is a promising method of PPNs management as it causes thermal death of the nematodes after exposure to high temperatures (D'Addabbo *et al.*, 2005). The effectiveness of soil solarisation in nematode management is affected by a number of factors including: weather and climatic conditions (Wang *et al.*, 2004; Wang and McSorley, 2008), intensity of sunlight, moisture, day length and soil structure (Coelho *et al.*, 2001). This method of nematode management can only be more efficiently applied with sufficient knowledge of the relationship between nematode survival and duration of exposure at different temperatures (D'Addabbo *et al.*, 2005).

3. MATERIALS AND METHODS

3.1. Survey of the distribution of *H. schachtii* and *H. cruciferae* in OSR-growing areas of the UK

3.1.1. Soil sampling, sample packaging and labelling

Survey sites were selected on the basis of OSR cropping during the season preceding sampling. Soil sampling was conducted after crop harvest in July and August 2009/2010 as this is the period when mature cysts are more likely to be detected even under low field infestation. Soil samples were taken from a depth of 20 cm using a cheese corer-type soil auger with a half cylindrical blade measuring 20 x 2.5 cm in a grid pattern across the entire field. Each soil sample was composed of 50 cores which were homogenised by hand mixing prior to taking a sub-sample of 2.5 kg. The sub samples were packed in cotton cloth bags which were labelled with details including: grower's name, field location, GPS coordinates and a five-year cropping history.

3.1.2. Cyst extraction and nematode species identification

Cysts were extracted from the soil according to the method of Trudgill *et al.* (1973) and the cyst nematode species present in each sample were identified using vulval cone top features following Mulvey (1972) and Jabbari and Niknam (2008). The J2 were hatched from the

eggs and the specimens were processed following De Grisse (1969). The specimens were examined under a Zeiss Axiovision microscope (Carl Zeiss MicroImaging, GmbH, Germany) at 1000x magnification. The images were taken using an A1 imager with AxioCam and Axiovision software 4.8 (Carl Zeiss MicroImaging, GmbH, Germany), using differential interference contrast, and projected onto an attached Dell computer Optiplex 780 (Dell Inc., Lodz, Poland).

3.2. Oilseed rape cultivar host status, yield and cyst nematode relationships

The experiments were conducted in a glasshouse and polytunnel using 15 cm-diameter plastic pots and seedlings of five winter OSR cvs Castille, Flash, DK Cabernet, ES Astrid and Catana. The OSR seedlings had been previously vernalised in a cold chamber at between 0 and 7°C for eight weeks prior to use, and each of the treatments included six replicates. Five *H. cruciferae* initial population densities of 0 (uninoculated control), 0.5, 2, 8, and 16 and *H. schachtii* population densities of 2, 4, 8, 16 and 32 eggs g⁻¹ of soil were used and sterile Kettering loam served as control.

After 12 weeks of incubation, plant growth and yield parameters, root analyses, final nematode population densities and multiplication rates (final population density/initial population density) were assessed following the method of Southey (1986) for each replicate and OSR cvs. The data were analysed using dose-response ANOVA in Genstat release 13.1 (VSN International Ltd, Hemel Hempstead, UK), and pair-wise comparisons were made using Tukey's multiple range test to identify significant differences between treatment means at a significance level of $P < 0.05$. The relationships between the final population densities, multiplication rates and initial population densities were ascertained using linear and group regression analyses.

3.3. Effect of temperature on the life cycle of *H. schachtii* infecting OSR

The effect of temperature on the life cycle of *H. schachtii* was assessed in water baths set at different temperatures ranging between 5 and 35°C. Experiments were conducted on hatching of J2, root invasion and duration of development using winter OSR cvs Castille and Flash, plastic pots measuring 6.5 cm in diameter and sterile silver sand. All the plants were inoculated with 200 freshly-hatched J2 of *H. schachtii* and initially incubated at an average of 24°C ($\pm 0.5^\circ\text{C}$) for 48 hours to allow the J2 to invade the root systems prior to being

transferred to the different water baths. The surface of the sand in each pot was insulated with 100 g of vermiculite and the water in the baths was covered with polystyrene to minimise heat loss. The plants were watered daily, nutrition was added weekly and temperature and relative humidity (RH) were recorded using data loggers.

The pots were checked daily starting two weeks after inoculation for hatching of the first J2. This was done by leaching 200 ml of distilled water through each pot and collecting the leachate after one minute for observation under a stereo microscope at 60x magnification. When the first-hatched J2 was detected in the leachate, the data loggers were removed for downloading of the temperature data and then replaced. Collection of J2 was continued for an extra week prior to harvesting of the plants for cyst extraction from the roots and pot sand. The temperature range within which the highest numbers of hatched J2 and cysts were observed was taken as the optimum temperature (T_o). The maximum temperature (T_m) for development was the highest temperature above T_o when there was no hatching of J2 of the second generation.

The data were analysed using dose-response ANOVA, whilst any treatment means showing significant differences were compared using Tukey's multiple range test. The cumulative percentage J2 hatch data were arcsine-transformed to remove skewness prior to performing ANOVA. The mean temperature above T_b from inoculation to hatch of the first J2 of the second generation, i.e. the accumulated heat units (AHU) in °C days was calculated from the formula:

$$AHU = \sum_{i=1}^n \frac{[(T1 + T2) - t]}{2}$$

where T1 and T2 are the minimum and maximum temperatures, respectively, on a given day, n is the number of days taken to complete the life cycle and t is the estimated base temperature (temperature below which no detectable development occurs). The duration of the life cycle at the different temperatures was taken as the period from inoculation to hatching of the first J2 of the second generation.

3.4. The number of generations completed during the growing season

The number of generations of *H. schachtii* completed on OSR during the growing season was investigated in outdoor pot experiments using two winter OSR cvs Flash and Castille and two spring OSR cvs Belinda and Heros. The experiments were established in September 2010 and March 2011 for the winter and spring OSR cvs respectively, and were

run until August 2011 coinciding with the harvest time in commercial OSR production. One hundred 26 cm-diameter pots were used for the winter and 60 for the spring OSR cvs. Each of the pots was filled with *H. schachtii*-infested field soil and four week-old OSR seedlings of either winter OSR cvs Flash or Castille or spring cvs Belinda or Heros were transplanted to each pot and arranged in five randomised complete blocks.

Soil temperature and RH recordings were taken at 10 cm-depth throughout the crops' growth period using a data logger which was placed in one of the pots. Soil samples from five plants per treatment were taken every month and processed prior to counting of the total numbers of each stage of development. The J2 of *H. schachtii* in two replicate sub-samples, each 200 g of the pot soil, were extracted utilising a modified method by Whitehead and Hemming (1965), whilst the J2 were estimated following the method of Southey (1986). The data were analysed using dose-response ANOVA and any treatment means showing significant differences were compared using Tukey's multiple range test. The AHU in degree days were calculated from the formula above.

4. RESULTS

The distribution of the samples collected from each county, the number of infested samples and the cyst nematode species present are shown in Table 1.

4.1. Cyst nematode detection and composition

Soil samples were obtained from 221 fields in 25 counties in OSR-growing areas of the UK (Table 1). Only two samples were obtained from Scotland both of which were negative for cyst nematodes and no samples were obtained from Wales or Northern Ireland. Cysts were detected in 14% of the samples (31 samples) (Table 1). The number of cysts recovered from the samples ranged between one and 74 cysts in 200 g⁻¹ of soil, 85% of samples had fewer than five cysts. The cysts were predominantly of the genus *Heterodera*, constituting 84% (26 samples) whilst a few *Globodera pallida* cysts were detected in 10% of the samples (3 samples). The *Heterodera* spp. detected belonged to two groups; the *schachtii* group and *H. avenae* complex, with *H. schachtii* being the most predominant. *Heterodera cruciferae* was not detected despite OSR being a host of this species.

4.2. Population density estimation and egg viability assessment

A large number of cysts did not contain any eggs whilst some of the cysts in which eggs were present had non-viable eggs. The viability of the eggs was ascertained following the

method of Shepherd (1962). Generally, low densities of cysts and eggs cyst^{-1} were observed in most samples with 85% of the samples having population densities less than 10 eggs g^{-1} of soil (Figure 2).

Table 1. Distribution of sampling areas, samples collected and number of infested samples

County	Number of samples	Number infested	Cyst species
Bedfordshire	7	3	H. s, H. a
Berkshire	1	0	-
Berwickshire (Scotland)	1	0	-
Cambridgeshire	18	3	H. s, H. a
Derbyshire	1	0	-
Durham	1	0	-
Essex	9	0	-
Gloucestershire	3	0	-
Herefordshire	9	1	H. s
Hertfordshire	10	0	-
Kent	14	1	H. s
Leicestershire	8	1	H. s
Lincolnshire	28	1	H. s
Nottinghamshire	18	1	H. a
Norfolk	29	10	H. s, H. a
Northamptonshire	1	0	-
Oxfordshire	6	1	H. a
Roxburghshire (Scotland)	1	0	-
Suffolk	17	0	-
Staffordshire	5	3	H. s, G. p
Shropshire	5	0	-
Warwickshire	10	4	H. s
Worcestershire	3	0	-
Wiltshire	4	0	-
Yorkshire	12	2	H. a
Total	221	31	

H. s = *Heterodera schachtii*, H. a = *Heterodera avenae* complex, G. p = *Globodera pallida*.

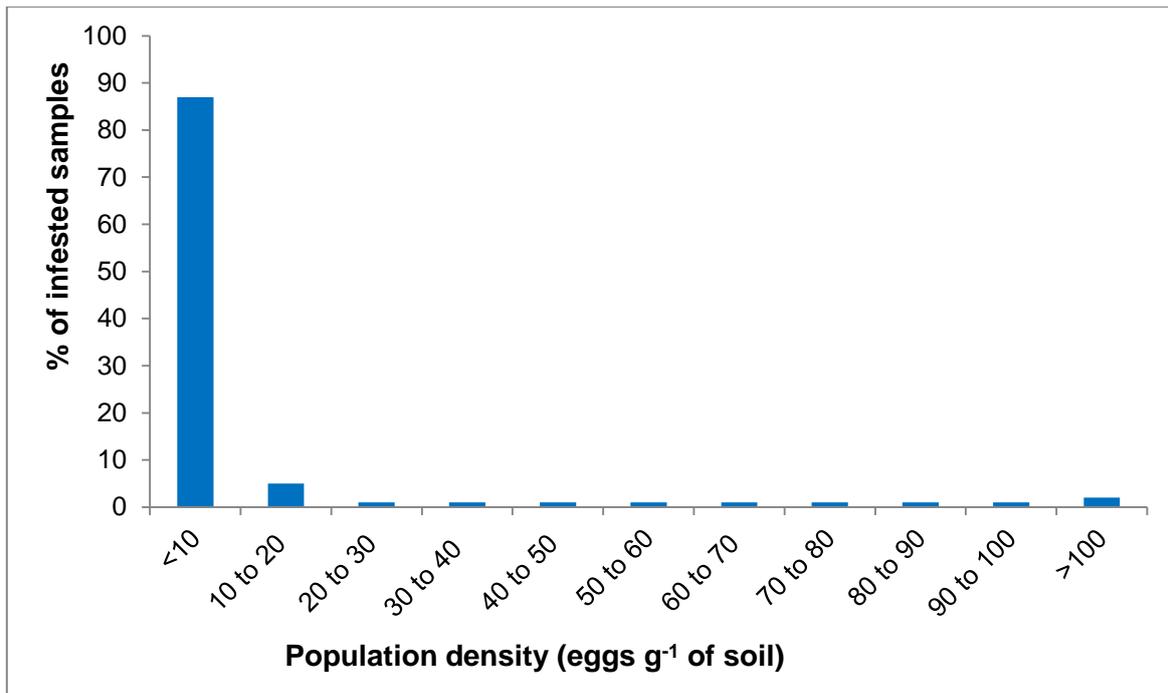


Figure 2. Cyst nematode infestation Pi's of the different soil samples.

4.3. OSR cultivars grown and rotation regimes

The five-year cropping history obtained from the growers revealed that a wide range of OSR cultivars were grown in the different regions of the UK. Rotation lengths practiced by growers varied between less than one year in two to more than one in four (Figure 3). The most commonly practised rotation length was one year in more than four (23% of growers), which is above the minimum recommended rotation length of at least one year in four. However, a large number of growers (39%) practise rotation lengths below one year in four whilst 17% did not specify (NS) the rotation lengths they practise (Fig. 3.2). A wide range of crops including winter wheat, winter barley, potatoes, spring beans, oats, spring peas, grasses, hemp and maize were grown in rotation with OSR, whilst 17% of growers grew sugar beet in rotation with OSR. However, there was no distinct relationship between population density and rotation lengths practiced.

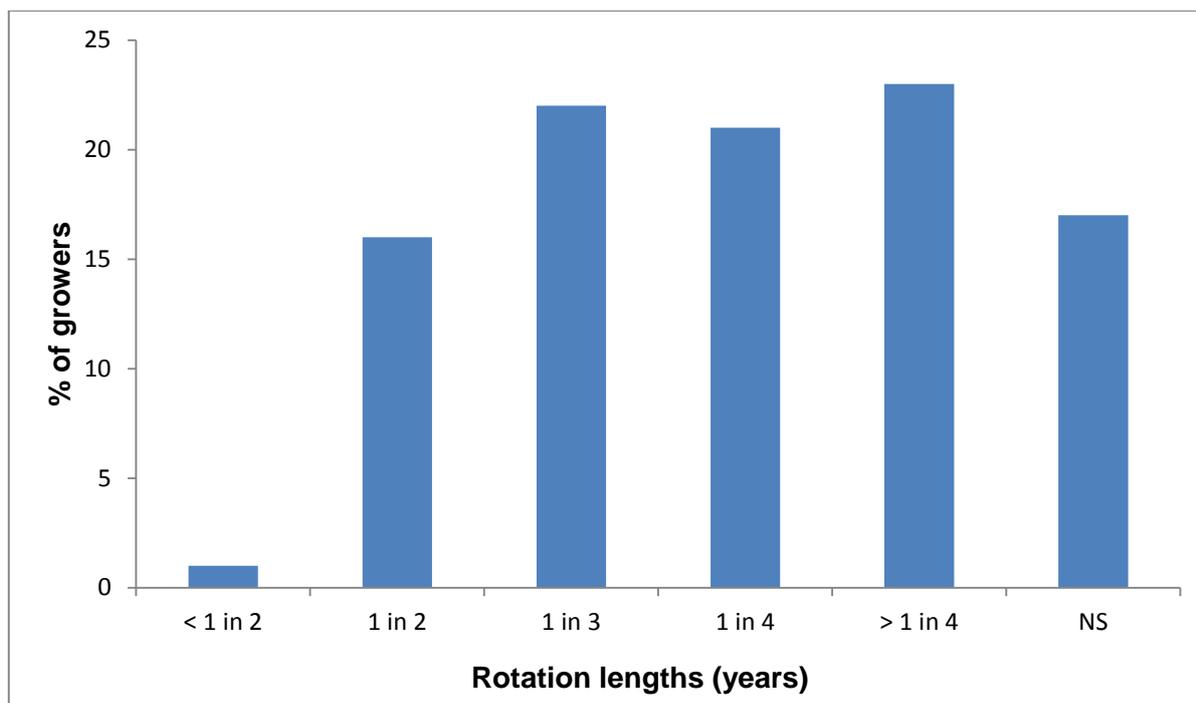


Figure 3. Percentage of OSR growers practising the different rotation regimes. NS = not **specified**.

4.4. Oilseed rape cultivar host status, yield and cyst nematode relationships

4.4.1. Relationship between Pf and Pi of *H. cruciferae*

Group regression analysis revealed significant ($P < 0.001$) positive linear relationships between final population densities and initial population densities of *H. cruciferae* in both winter OSR cvs Flash and Castille, accounting for 95.1% of the observed variation (Figure 4). Although there was an overall increase in final population densities of *H. cruciferae* with increasing initial population densities in both cvs Flash and Castille, the increase was below the respective initial population densities (Figure 4). The increase in final population densities of *H. cruciferae* was greater in cv. Flash than cv. Castille. Cultivar Flash produced the highest final population densities of approx. 0.5 eggs g⁻¹ of soil compared to that of approx. 0.2 eggs g⁻¹ of soil produced by cv. Castille at an initial population density of 16 eggs g⁻¹ of soil (Figure 4).

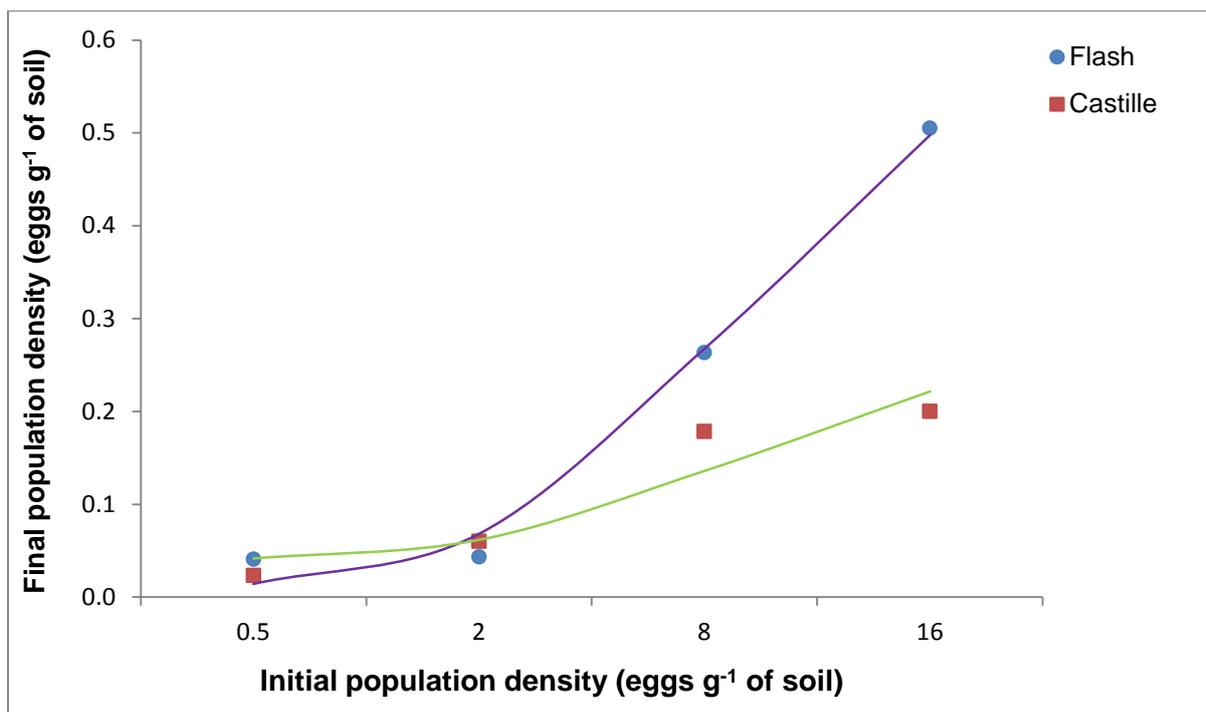


Figure 4. The relationship between final population densities and initial population densities of *H. cruciferae* and OSR cvs Flash and Castille. Values are means of six replicates. The lines represent the predicted functions calculated by fitting the following exponential regression models: Flash: $y = 1.767 - 1.771(0.9794^x)$; Castille: $y = 0.6939 - 0.6590(0.9794^x)$.

$P < 0.001$; Percentage variance accounted for = 95.1%; SEM (25df) = 0.034.

4.4.2. Relationship between multiplication rate and initial population density

Group regression analysis between the final/initial population density ratios (multiplication rates) of *H. cruciferae* on cv. Flash and cv. Castille and the different initial population densities revealed significant ($P < 0.001$) negative linear relationships (Figure 5). Multiple comparisons of the treatment means using Tukey's multiple range test revealed significant ($P < 0.005$) differences between multiplication rates of cv. Flash and cv. Castille and the different initial population density of *H. cruciferae* (Figure 5). The decline in multiplication rates was slower in cv. Flash (approx. 0.05) than in cv. Castille (approx. 0.04) between initial population densities of 0.5 and 16 eggs g⁻¹ of soil (Figure 5). Cultivar Flash had a higher overall multiplication rate than cv. Castille.

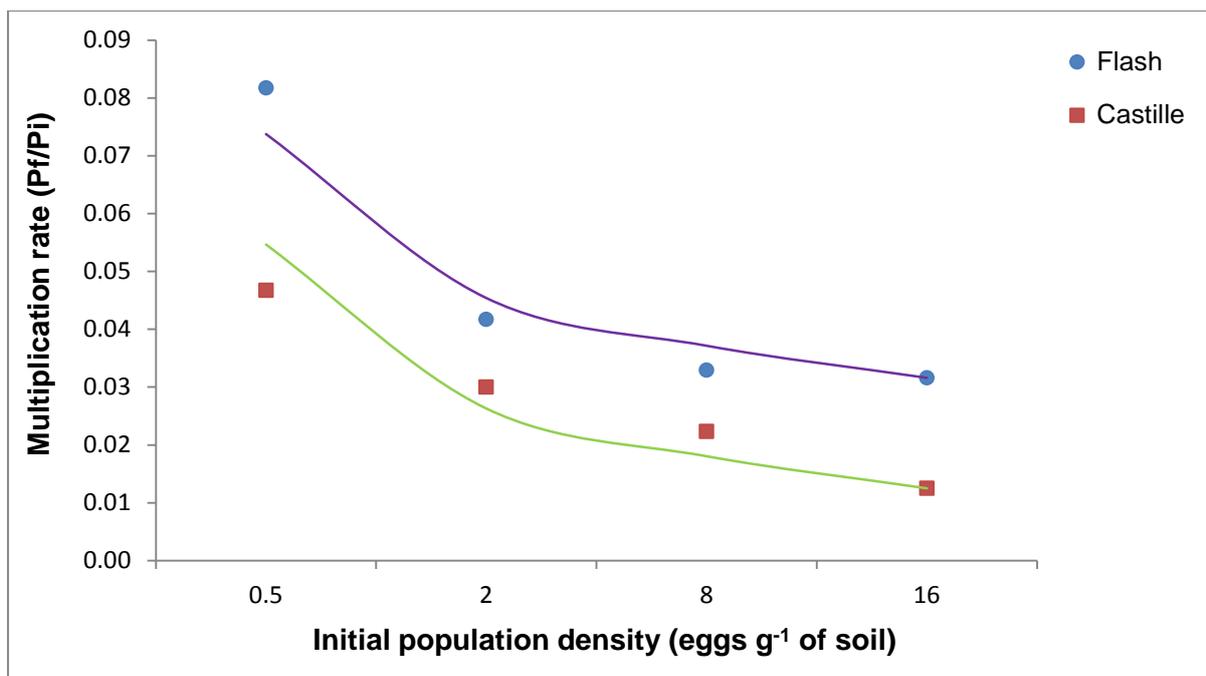


Figure 5. The relationship between multiplication rates (final population density/initial population density) of *H. cruciferae* and initial population densities on OSR cvs Flash and Castille. Values are means of six replicates. The lines represent the predicted functions calculated by fitting the following line plus exponential regression models: Flash: $y = 0.04270 + 0.06194(0.257^x) - 0.0006936x$; Castille: $y = 0.02360 + 0.06194(0.257^x) - 0.0006936x$. $P = 0.005$; Percentage variance accounted for = 85.3%; SEM (25df) = 0.006.

4.4.3. Effect of *H. schachtii* on OSR damage and yield

Relationship between final and initial population densities of *H. schachtii*

Whilst the final population densities of *H. schachtii* increased with increasing initial population densities in all the OSR cvs, they were significantly ($P < 0.001$) lower than the respective initial population densities (Figure 6). Cultivar Flash produced the highest overall final population density of *H. schachtii* at all the initial population densities investigated, with the highest final population density of approx. 9 eggs g⁻¹ of soil observed at the highest initial population density of 32 eggs g⁻¹ of soil (Figure 6). This was followed by cv. DK Cab which produced the second highest final population density of approx. 8 eggs g⁻¹ of soil at the highest initial population density of 32 eggs g⁻¹ of soil. The lowest final population density of approx. 0.5 and 1.8 eggs g⁻¹ of soil were observed in cvs DK Cab and Castille, respectively, at initial population densities of 4 and 8 eggs g⁻¹ of soil, respectively (Figure 6). The increase in final population density was slow, remaining almost constant between 2 and 4 eggs g⁻¹ of soil in all the cvs but increased rapidly between 4 and 32 eggs g⁻¹ of soil except in cv. Castille whose final population density declined by approx. 1 egg g⁻¹ of soil between 16 and 32 eggs g⁻¹ of soil (Figure 6).

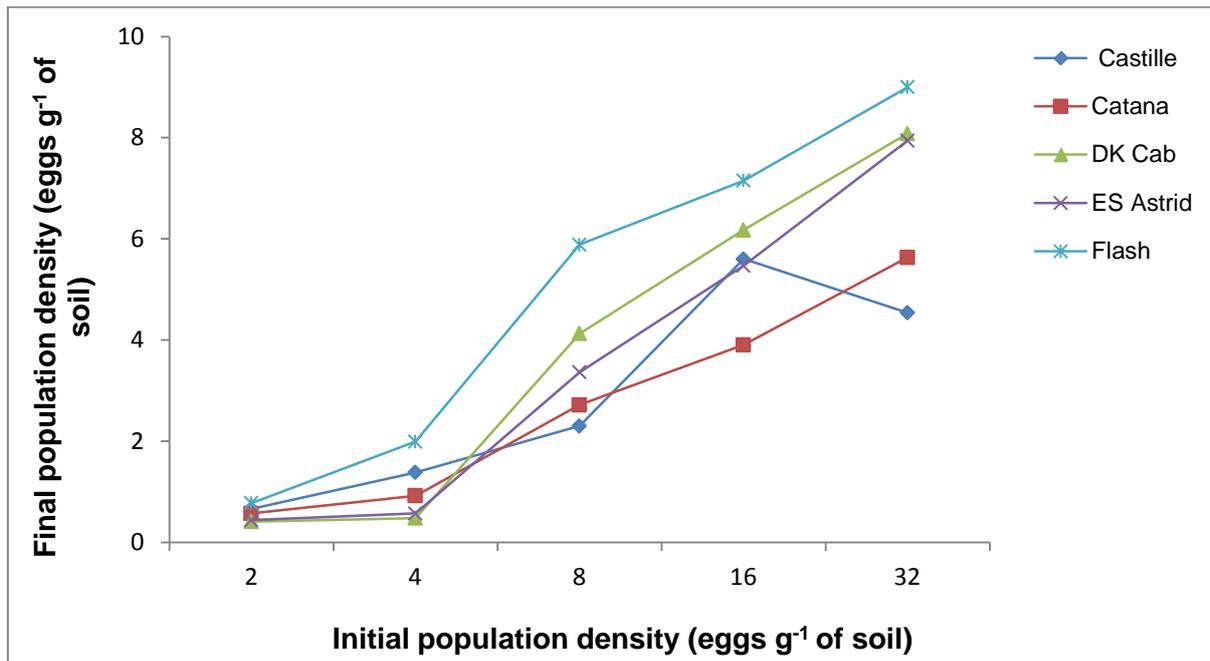


Figure 6. The relationship between final and initial population densities of *H. schachtii* on five winter OSR cvs. Values are means of six replicates. $P < 0.001$; SEM (145df) = 0.249.

Relationship between multiplication rate and initial population density of H. schachtii

Dose response ANOVA revealed significant ($P < 0.001$) negative linear relationships between multiplication rates and initial population densities of *H. schachtii* for the different cultivars. The most rapid decline in the multiplication rate was observed between 2 and 4 eggs g⁻¹ of soil in all the OSR cvs (Figure 7). The highest multiplication rates of approx. 15.8 and 11 were observed in cvs Flash and Castille, respectively at 2 eggs g⁻¹ of soil. The lowest multiplication rate of approx. 1 was observed in all the cultivars at 32 eggs g⁻¹ of soil (Figure 7). A gradual decline in multiplication rate was observed in all the OSR cvs remaining almost constant between 4 and 32 eggs g⁻¹ of soil, and did not differ ($P > 0.05$) significantly between the cvs (Figure 7).

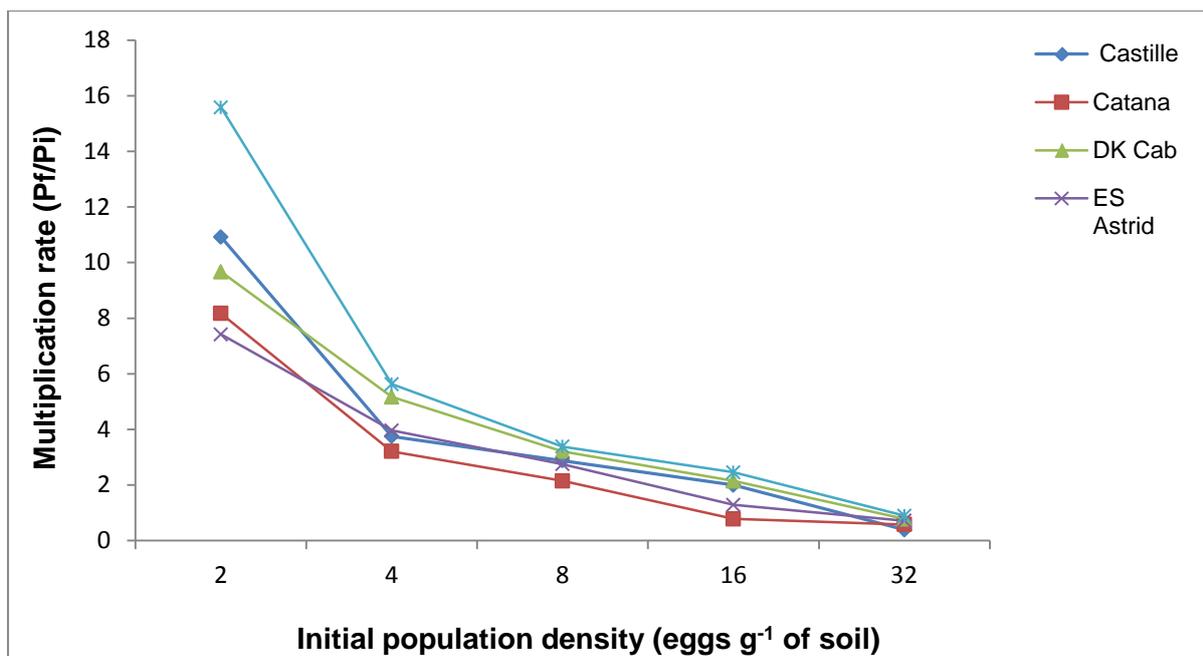


Figure 7. The relationship between multiplication rates and initial population densities of *H. schachtii* on five winter OSR cvs. Values are means of six replicates. $P < 0.001$; SEM (145df) = 0.249.

4.5. Effect of temperature on the life cycle of *H. schachtii* infecting OSR

Temperature significantly affected the duration of the life cycle. The first-hatched *H. schachtii* J2 of the second generation were detected after six weeks at the lowest temperature of 5.0°C compared with only three weeks at 20.5 and 27.8°C (Table 2). The highest numbers of J2 and cysts were observed at 27.8°C followed by 20.5°C (Table 2) indicating that this was the T_o range for development of this *H. schachtii* population. The duration of the life cycle was least at 20.5°C (21 days) and 27.8°C (23 days) and increased both above and below these temperatures to 38 days at 37.5°C and 42 days at 5.0°C (Table 2). Very few J2 and cysts developed at 5.0°C and 37.5°C and fewer AHU were required to complete development at lower temperatures. The AHU above the T_b of 5.0°C required for completion of the life cycle from J2 to J2 at each temperature are shown in Table 2. The least AHU of 203°C days was required for completion of the life cycle at the lowest temperature of 5.0°C and the highest of 1,406°C days was required at the highest temperature of 37.5°C (Table 2).

As temperature increased, the AHU required to complete the life cycle increased from 203°C days at 5.0°C to 1,406 at 37.5°C (Table 2). No significant ($P = 0.65$ and $P = 0.07$) differences were observed between J2 and cysts at 5.0 and 37.5°C, respectively, in both cvs Flash and Castille. However, significant ($P = 0.01$ and $P < 0.001$) differences were observed

between the latter two parameters at 5.0°C and 37.5°C, respectively, and the rest of the temperatures (Table 2). At 20.5 and 27.8°C, the numbers of J2 and cysts g⁻¹ of root were 10 to 15 times higher than those at 5.0°C and 37.5°C (Table 2). No significant ($P > 0.05$) differences in the number of J2 g⁻¹ of root were observed in cv. Flash at 20.5°C (47.3%) and 27.8°C (52.7%) but significant ($P = 0.01$) differences were observed in cv. Castille (42% and 58%) at both temperatures respectively (Table 2). Significantly ($P < 0.05$) more cysts of the second generation g⁻¹ of root were observed in cv. Flash than cv. Castille at 27.8 and 32.2°C, suggesting cv. Castille is a poorer host of *H. schachtii* than cv. Flash (Table 2).

Table 2. Effect of temperature and OSR cultivars Flash and Castille on duration of development of *H. schachtii* from J2 to J2.

Temp (°C)	OSR cultivar	J2	Cysts	Life cycle duration (days)	AHU (°C days above 5°C)
5.0	Flash	1.300 ^a	1.200 ^a	42	203
	Castille	1.100 ^a	1.400 ^a		
10.1	Flash	5.500 ^c	2.100 ^a	40	398
	Castille	5.700 ^c	2.700 ^a		
20.5	Flash	10.700 ^e	10.300 ^c	21	424
	Castille	9.100 ^d	12.700 ^{cd}		
27.8	Flash	11.900 ^{ef}	15.400 ^d	23	624
	Castille	12.600 ^f	11.400 ^c		
32.2	Flash	2.900 ^b	9.900 ^c	34	1,143
	Castille	2.900 ^b	6.400 ^b		
37.5	Flash	1.400 ^a	1.000 ^a	38	1,406
	Castille	1.200 ^a	1.000 ^a		
SEM (df = 71)		0.305	0.597		
CV (%)		17.5	30		
P value		0.01	<0.001		

Any two means in the same column which have a letter in common are not significantly different at $P < 0.05$ according to Tukey's multiple range test. Values are means of juveniles and cysts g^{-1} of root for ten replicates.

4.6. The number of generations of *H. schachtii* completed on OSR during the growing season

4.6.1. Second stage juveniles in the soil

Second stage juvenile counts from the monthly soil sample analyses revealed that the winter OSR cv. Castille and the two spring cvs Belinda and Heros produced two peaks of J2, whilst the winter cv. Flash produced three peaks during the growing season (Figures 8 and 9). Second stage juveniles were not observed in the soil during the first 120 days (30th September to 30th January) after planting of winter OSR cvs Flash and Castille (Figure 8). The first J2 peaks were observed in the soil 148 days after planting (28th February) in cv. Castille and 178 days after planting (30th March) in cv. Flash (Figure 8). The second peaks of J2 were observed in the soil 238 days after planting (30th May) in cv. Flash and 268 days after planting (30th June) in cv. Castille (Figure 8). A third J2 peak was produced in the soil of cv. Flash 298 days after planting (30th July) (Figure 8).

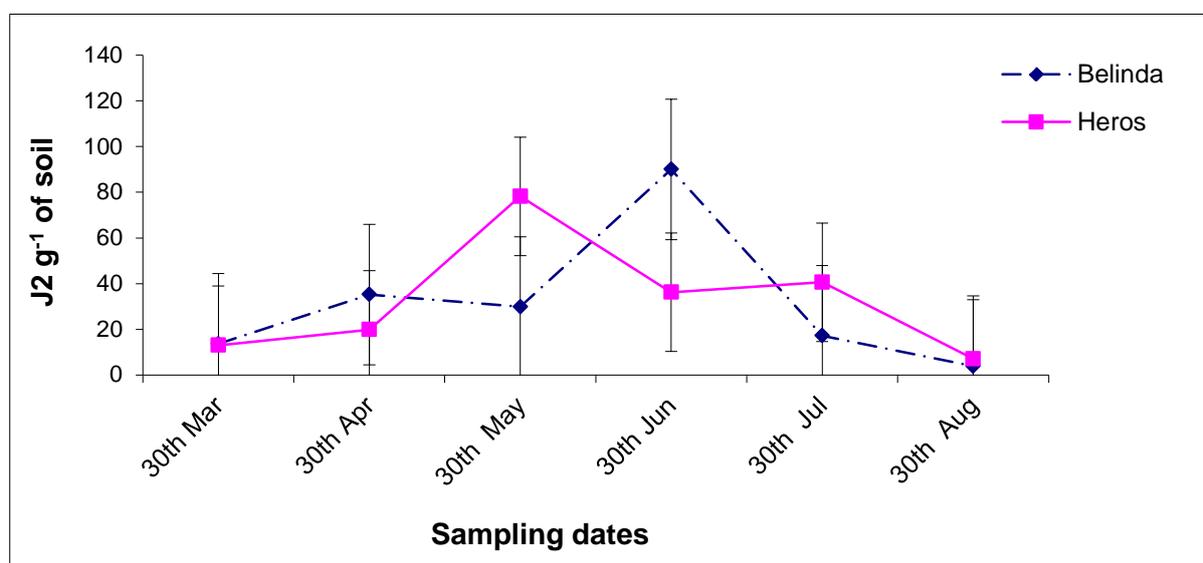


Figure 8. The number of generations of *H. schachtii* completed on spring OSR cvs Belinda and Heros during the growing season. Values are means of five replicates. Vertical bars represent standard errors of the means.

The mean numbers of J2 in the two peaks of cvs Flash and Castille varied significantly ($P < 0.001$) with the first peaks having the highest of approx. 60 and 62 J2 g⁻¹ of soil in cvs Castille and Flash respectively (Figure 8). The second J2 peaks contained approx. a half (34 and 36 J2 g⁻¹ of soil) in cvs Flash and Castille respectively (Figure 8). However, the third peak observed in cv. Flash contained approx. a quarter (16 J2 g⁻¹ of soil) of the number of J2 observed in the first and a half of those observed in the second peaks (Figure 8).

The first peaks of J2 were observed in the soil of spring cultivars 61 days after planting (30th April) in cv. Belinda and 91 days after planting (30th May) in cv. Heros (Figure 9). The second J2 peaks

were observed 121 days after planting (30th June) in cv. Belinda and 151 days after planting (30th July) in cv. Heros (Figure 9). The highest of approx. 90 J2 g⁻¹ of soil and the least of approx. 35 J2 g⁻¹ of soil J2 numbers were observed in the soil of cv. Belinda on 30th June and 30th April respectively (Figure 9). Both spring and winter OSR cvs produced J2 peaks in the soil from samplings conducted on 30th May (Flash and Heros) and 30th June (Castille and Belinda), whilst the last J2 peaks were observed on 30th July in both the winter and spring OSR cvs Flash and Heros (Figures 8 and 9).

Generally, spring OSR cvs produced higher total numbers of J2 (228 J2 g⁻¹ of soil) than winter cvs (192 J2 g⁻¹ of soil) (Figures 8 and 9).

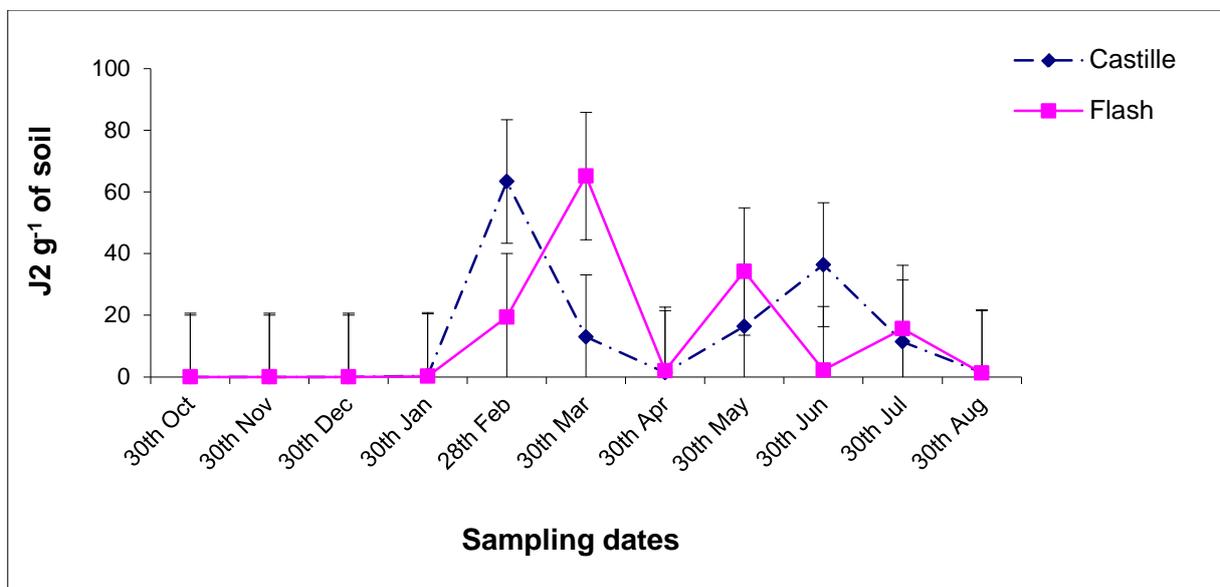


Figure 9. The number of generations of *H. schachtii* completed on winter OSR cvs Flash and Castille during the growing season. Values are means of five replicates. Vertical bars represent standard errors of the means.

5. DISCUSSION

The rationale for this research was premised on the lack of current information on the status of cyst nematodes infecting OSR in the UK. In addition, there had hitherto not been a nationwide survey conducted in OSR-growing areas of the UK to establish the distribution and population densities of OSR cyst nematodes. This is with exception of a few localised surveys which were conducted in brassica vegetable-growing areas in the 1980s. The absence of breeding programmes against OSR cyst nematodes in the UK as well as the lack of approved chemical nematicides for use in the management of these cyst nematodes were the impetus for this research.

The OSR cyst nematode survey reported here is the first nationwide survey conducted in UK OSR-growing areas. The findings from the survey indicated that the OSR cyst nematode species *H.*

schachtii was limited in distribution, whilst *H. cruciferae* was not detected in the surveyed OSR-growing areas. The population densities of *H. schachtii* were also very low in most of the infested fields which is contrary to what had been predicted by previous researchers that these nematode species were widespread in the UK. However, the observed low densities pose a potential risk of high population density build-up in the future as multiplication rates are highest under these conditions, which is attributed to limited intra-specific competition. The survey results also indicated that a high percentage of growers practised rotation lengths lower than the minimum recommended of at least one year of OSR in four of non-host crops of OSR cyst nematodes. This, coupled with the production of sugar beet in rotation with OSR, has implications for maintaining population densities of the OSR cyst nematodes above damage threshold levels. The soil conditions of infested fields are also crucial as damage to OSR by *H. schachtii* and *H. cruciferae* can be high even when the population densities are low as reported by Bowen (1988).

The absence of *H. cruciferae* from soil samples obtained during the present survey may either be because the population densities were too low to be detected or this cyst nematode species may have been outcompeted by *H. schachtii* since both species often occur in the same areas (Caswell-Chen *et al.*, 1992). These arguments are supported by Bowen (1988) who observed that *H. cruciferae* populations became extinct when their population densities were low and this was aggravated by their co-existence with *H. schachtii*. Bowen (1988) also reported that *H. cruciferae* was more economically important in brassica vegetables such as cabbage and cauliflower than in other host crops such as OSR, which may partly explain why it was not detected during the present survey.

In addition, since the resistance status of current commercial UK OSR cvs is unknown, it is possible that some or all of these cvs may be resistant to *H. cruciferae* leading to selection against *H. cruciferae* in favour of *H. schachtii*. This may have led to the observed dominance of *H. schachtii* as revealed by the survey.

Another possibility could be that as *H. cruciferae* multiplies better under cooler conditions as reported by Bowen (1988), this species may be more abundant in the northern and north eastern parts of the UK. This is because the northern and north eastern areas are cooler than the eastern parts of the country (UK Met Office, 2011) where most OSR is grown. This argument is supported by the high population densities of *H. cruciferae* which were detected in over 70% of brassica vegetable-growing areas of Lincolnshire by previous researchers. However, the high percentage of *H. cruciferae* detected by Winfield *et al.* (1970) in Bedfordshire, which is more south and warmer, may be due to agronomic practices rather than temperature. It would therefore be helpful to conduct a survey in brassica vegetable-growing areas in order to ascertain if *H. cruciferae* is still

present and/or abundant in these areas. These areas were not covered by the present survey because they were outside the scope of the research.

It was also discovered through interaction with OSR growers during the survey that most of them were unaware of the existence of OSR cyst nematodes. Although some growers practiced long rotation lengths, the main reason for practising long rotation lengths was yield improvement of subsequent OSR crops and not as a result of grower awareness of the potential damage to OSR caused by cyst nematodes. Since a wide range of OSR cvs are grown commercially, yet their resistance and/or tolerance status is unknown, it was difficult during the present survey to directly link the observed low nematode population densities to OSR cultivar resistance although this may be a possibility. However, since potatoes are grown in rotation with OSR, the nematicides used to control PCN during potato production may have also played a role in maintaining the observed low population densities of *H. schachtii*. This argument is underpinned by the fact that a large number of cysts which were recovered from the different soil samples were empty, whilst a substantial number of them contained non-viable eggs. The low *H. schachtii* population densities and the limited species diversity observed from the present survey imply that there are no concerns at the moment by OSR growers arising from restricted nematicide use in crop protection as a result of the implementation of the EU directives 91/414/EEC and EC 1107/2009.

Most of the experiments reported under the present research were conducted under glasshouse and polytunnel conditions. This was because there were no known fields with heavy infestations of the two OSR cyst nematode species, *H. schachtii* and *H. cruciferae* at the time of commencement of the research. The findings from the survey have further confirmed that fields which are heavily-infested with the two OSR cyst nematode species were very limited and none of them had *H. cruciferae*. It was thus difficult to obtain heavily-infested field soil with *H. cruciferae* for use in experiments which is why most of the experiments were conducted using *H. schachtii*. However, the main challenge associated with field experimentation involving cyst nematodes is obtaining fields with uniform population densities as cyst nematodes often occur in aggregations within fields. Blocking of treatments on the basis of population densities alone would not solve the problem of large variations within treatments as observed by Whitehead *et al.* (1994). Reduced within-treatment variation can be achieved when other environmental factors, including soil fertility and gradient of the area intended for siting the experiment, are put into consideration.

The results from the temperature experiments showed that the life cycle of *H. schachtii* was greatly dependent on temperature, the duration having ranged between three and six weeks at different temperatures. At least two generations of *H. schachtii* were completed on winter OSR cvs Flash and Castille and spring cvs Belinda and Heros. However, in view of the rising soil temperatures in the UK as reported by Carter *et al.* (2000) and Subedi and Fullen (2009), there is high potential for

multiple cyst nematode generations to be completed on OSR during the growing season, which could lead to high cyst nematode population density build-up on OSR in the near future. High population densities may pose a potential threat of damage and yield loss to OSR. Therefore, information on temperature aspects of cyst nematode development such as that obtained from the present study can be useful in monitoring biological processes of OSR cyst nematodes. This can guide growers on the timely implementation of agronomic practices including: drilling and application of nematode management measures before the nematodes build up to damagingly high levels, which can help minimise current and future crop damage and yield loss.

The knowledge of temperature requirements for nematode development has been utilised by a number of researchers in other countries including Ferris *et al.* (1978) who used it in the USA to develop a population dynamic and yield loss model for *Meloidogyne arenaria*, whilst Tiilikkala *et al.* (1988) used similar information in Finland to assess whether *M. hapla* could become established there. Langeslag *et al.* (1982) used temperature information in the cultural control of PCN in potato fields in Germany. There has also been a proposal by Trudgill (1995b) to develop cold-adapted strains of entomopathogenic nematodes in the UK which would work efficiently at temperatures below 15°C, whilst Bongers (1990) in the Netherlands suggested a method of calculating nematode maturity indices using the AHU concept.

However, in the UK, hardly any reports on the use of temperature information in cyst nematode population dynamics predictions and management are available which may be because research on this aspect is limited. Therefore, the temperature information generated from the present study is a useful, novel contribution to this area especially with regard to current OSR cvs and the *H. schachtii* population used. This information could potentially form a basis for continuous monitoring of the field population dynamics of *H. schachtii* and other cyst nematodes during the growing season of OSR in the UK.

The results on OSR cultivar host status constitute the first report on this aspect with respect to current commercial winter OSR cvs and *H. schachtii* and *H. cruciferae* in the UK. The results imply that there are differences in the resistance and tolerance status of winter OSR cvs. The poor performance of cv. Flash, despite it being a restored hybrid may be attributed to the growing conditions which may have prevented it from fully expressing its inherent heterosis or its tolerance of cyst nematode damage could have been compromised during the breeding process. This is because genes responsible for some cv. attributes are often lost during breeding especially when they are not directly associated with the characters being selected for (Cooke and Evans, 1987). In addition, since UK OSR cvs are not specifically bred for resistance against OSR cyst nematodes, the anticipated heterosis characteristics in cv. Flash with regard to cyst nematode resistance may be lacking, making it more susceptible to cyst nematode damage than the conventional cvs. This

information can form a basis for informed OSR production decisions such as the choice of suitable OSR cvs to grow in cyst nematode-infested areas.

5.1. Practical messages from the results for OSR growers

The findings obtained from this research can be useful for predicting future field populations of *H. schachtii* and *H. cruciferae* using population dynamics models. This information can be used to inform the timely application of management measures in order to maintain the nematode population densities below economic threshold levels in infested areas. For instance the survey results can help guide OSR growers in the selection of less infested or uninfested sites for production of host crops of the two cyst nematode species, which would minimise subsequent crop yield losses.

The information from the interaction between *H. schachtii* and *H. cruciferae* with OSR indicated that there are differences in the levels of resistance and tolerance of current commercial winter OSR cvs to these cyst nematode species in the UK. The host status to *H. schachtii* of the winter OSR cvs investigated in the present study was in the order Flash, DK Cab, ES Astrid, Castille and Catana, from the most to the least susceptible. The results also revealed that OSR hybrid cvs Flash and Belinda did not manifest hybrid vigour with respect to cyst nematode resistance, as they were most damaged and favoured more cyst nematode multiplication than the conventional winter OSR cvs DK Cab, ES Astrid, Castille, Catana, and the spring cv. Heros. This information can be used as a basis for the choice of more resistant OSR cvs (once these become available through breeding), to grow in *H. schachtii*-infested areas.

The results obtained from the temperature experiments can be integrated with the knowledge that the mean annual temperature rise in the UK will be 2 to 3°C by 2050 as predicted by Carter *et al.* (2000). This implies that the soil will become warmer in the winter and over the entire OSR-growing period. The rise in soil temperatures will apply a selection pressure to the *H. schachtii* populations in the field and presumably *H. cruciferae*. The selection pressure may lead to a gradual upward shift in the T_0 range for development of these cyst nematodes as they adapt to changes in temperature. The duration of the life cycles will become shorter as the nematodes will tend to develop faster within the T_0 range. This will have implications for the potential development of more generations of these cyst nematodes on OSR during the growing season, leading to increased crop losses.

Equipped with this information, OSR growers will be in a better position to appropriately time crop agronomic practices. The AHU concept can be practically used by UK OSR growers as a heuristic technique in the management of cyst nematodes. This can be done by using a degree day calculator such as the one developed by the Water and Atmospheric Resources Monitoring

Program (WARM) of the Illinois State Water Survey, University of Illinois (Anon, 2012c). Since the AHU or growing degree days is a measure of the nematodes' phenological development; it can be used to estimate the duration of cyst nematode life cycles and guide OSR growers in the choice of management decisions and the timing of application of management measures.

Every cyst nematode species has specific base (T_b) and T_m values for development which are known from literature. Hence the AHU can be calculated from the AHU formula described previously using daily records of the minimum (T_{min}) and maximum (T_{max}) temperatures over the developmental period of the cyst nematode species being investigated. The output from the calculation can then be used to estimate the time when the most damaging or vulnerable life stage of the nematode occurs, which will inform the appropriate timing of control measures. However, in order to accurately estimate the duration of a particular cyst nematode species' life cycle, OSR growers would need to choose a starting point to serve as a reference point for the daily recording of the T_{min} and T_{max} , which can be a calendar date or a specific biological event called a biofix. The biofix can be the time when, say, the first nematode J2 is detected in the soil or roots of host plants, and can be used as a basis for continuous monitoring of the progress of development of the cyst nematode species until the life cycle is completed.

Oilseed rape growers can also apply the AHU concept by growing cold-adapted crop cvs which can be sown in the autumn in cyst-nematode-infested soil, when soil temperatures are unfavourable for cyst nematode development. The crops would then be able to establish themselves when the nematodes are still dormant and by the time the soil temperatures rise in the spring and become favourable for J2 hatch, the plants will have developed stronger roots and will be less prone to damage which would subsequently reduce yield loss.

Oilseed rape growers can also apply the AHU concept by growing quick-maturing or early crop cvs, which require fewer AHU to complete their growth cycles before cyst nematodes reach damaging levels. This practice can help to reduce cyst nematode-induced crop damage and yield loss.

The AHU concept can also be used to monitor disease/pest out breaks for early warning purposes. This is because as a result of climate change, the rates of development of pests and pathogens are bound to increase when environmental temperatures are high above the T_b for long periods during the growing season. Oilseed rape growers would then be able to appropriately schedule growing of OSR during periods when the cyst nematode population densities are anticipated to be relatively low. The resultant benefit would be reduced cyst nematode damage and subsequent reduction in cyst nematode management costs.

Based on the findings from the survey and the knowledge of the life cycles of cyst nematodes, it is suggested that OSR growers should maintain sufficiently long rotations of at least one OSR in four of non-host crops of OSR cyst nematodes such as cereals.

The production of sugar beet in rotation with OSR by some growers as observed from the survey is discouraged, as this could increase population densities of *H. schachtii* which could damage subsequent OSR crops.

Oilseed rape growers should also be cautious whilst moving plants and soil during field operations as cyst nematodes could easily be spread from infested to uninfested areas.

The above measures, once cautiously implemented either individually or concertedly, would reduce the spread of cyst nematodes to infested areas, and maintain population densities in infested fields at below damage threshold levels.

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